

HETEROSIS FOR SEED YIELD, ITS COMPONENTS AND WILT RESISTANCE OVER ENVIRONMENTS IN CASTOR (*RICINUS COMMUNIS* L.)

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ABSTRACT

Investigation of 56 genotypes including 10 parents, 45 hybrids (developed by diallel mating design excluding reciprocal) and 1 standard check GCH 7 to determine the extent of heterosis for seed yield, its components and wilt resistance over environments. The data on heterobeltiosis revealed that, cross SKI 341 x GC 3 and SKI 341 x SPS 43-3 were given consistent performance for seed yield per plant in all the environments. The cross MCI 108 x SKI 341 and SKI 341 x SPS 43-3 were exhibited significant positive standard heterosis in all the environments for seed yield per plant. The cross which found significant for seed yield per plant were also found resistance to wilt. Magnitudes of heterosis vary from character to character and cross to cross. In general, for seed yield/plant across the locations magnitude of desirable heterosis was high over better parent but low over standard checks. For developing high yielding and earlier maturing genotypes, selection of crosses on the basis of per se performance with considerable per cent heterobeltiosis and standard heterosis would be more desirable.

KEYWORDS: *Castor, Diallel, Heterosis, Ricinus Communis L*

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INTRODUCTION

Castor, *Ricinus communis* L with $2n=20$, belongs to the family Euphorbiaceae and it is indigenous to eastern Africa and most probably originated in Ethiopia.; It is the only oilseed crop of India which earns about thousands of crore rupees of foreign exchange to the country every year. Though crop has wider adaptability and well responsive to irrigation and nutrients, its cultivation is mostly confined to arid and semi-arid regions of the country. The phenomenon of heterosis has proved to be the most important genetic tool in enhancing yield of often cross pollinated and cross pollinated crops in general and castor in particular. The exploitation of heterosis on commercial scale in castor is the major breakthrough in the enhancing its productivity. For genetic improvement, selection of superior parents is one of the important step for development of better hybrids. Though per se performance is taken as selection criterion, proper information on magnitude of heterosis, combining ability and gene action for seed yield and component traits involved in the inheritance in different parents and their crosses would be more helpful in selecting appropriate parents and desirable cross combinations for commercial exploitation of hybrid vigour (Dangaria et al; 1987). The present study was therefore undertaken, to determine the extent and mechanism of heterosis under different environments level in castor and ultimately, to isolate inbreds which can be more responsive to all environments.

MATERIAL AND METHODS

The experiment material consisted of 45 hybrids, generated by crossing 10 diverse inbreds (MCI-108, SKI-341, GC-3, SH-41, SKI-337, SKI-346, 1379, SPS 35-50, SPS 43-3 and SKI-307) in half diallel fashion and GCH-7, the most successful hybrid as standard check. The parents selected were elite genotype of the crop developed by pedigree breeding at Main Castor Mustard Research Station, S.D. Agricultural University, Sardarkrushinagar, Mandore, Rajasthan, JAU, Junagadh and at Directorate of Oilseeds Research, Hyderabad (Table 1). The resulting hybrids along with parents and standard check were evaluated at Main Castor-Mustard Research Station, S.D. Agricultural University, Sardarkrushinagar in Randomized Block Design under three replications over four environments. The crop is well sensitive to wilt and it has the potential for successful cultivating in pre-rabi season too. Therefore to study the response of the crop at varying levels of wilt incidence and sowing time, environments were created by two different normal sowing plot and wilt sick plot, and two different sowing dates (15th August and 15th September). Thus investigation was taken up in four environments viz; E₁: Sowing date 15th August, Normal plot sowing; E₂: Sowing date 15th August, wilt sick plot sowing; E₃: Sowing date 15th September, Normal plot sowing; E₄: Sowing date 15th September, wilt sick plot sowing. Each genotype was sown in a single row of fifteen dibbles. Parents, hybrids and standard check were randomized separately in each replication and environment too. The distance between two rows was 120 cm, while between two plants within a row was 60cm. The crop was raised under irrigated condition and all the agronomic practices and plant protection measures were adopted for raising a normal crop. Five competitive plants from each treatment in each replication and environment were, selected randomly and tagged, used for recording observations on seed yield per plant (g), day to 50 % flowering of primary raceme, days to maturity of primary raceme, plant height upto primary raceme (cm), no. of nodes upto primary raceme, no. of branches/plant, length of primary raceme, no. of capsules on primary raceme, 100-seed weight and wilt incidence. The analysis of variance for each trait was calculated as per Panse and Sukhatme (1978). The superiority of hybrids was estimated over better parents as heterobeltiosis and as standard heterosis over check GCH-7 according to the method of Fonseca and Patterson (1968).

RESULTS AND DISCUSSIONS

Analysis of Variance

The analysis of variance for experimental design (Table 2) revealed considerable genetic variation among the parents and hybrids for all the traits evaluated in individual as well as pooled over environments. This indicated that the experimental material selected and hybrids generated possessed sufficient genetic diversity. Comparison of mean squares due to parents vs. hybrids across the environments was found to be highly significant for most the traits which suggested that parental group as a whole was quite different from their F₁s thus revealing the presence of heterosis for all the traits under investigation. The results revealed that mean square values due to genotypes were significant for all the characters in each individual environment as well as pooled over the environments, indicating the presence of sufficient amount of genetic variability in the materials study under individual environments. The variances for parents were significant for all the characters across the environments. These findings indicated that significant variability was existing among the parents for majority of the traits under study. The mean squares of hybrids were found significant for all the characters in individual environment as well as pooled over the environments. The analysis of variance further revealed that parents vs hybrids differed significantly for all the characters in all four environments and pooled over the environments excepts, for days to 50 % flowering, number of nodes upto primary raceme and plant height upto primary raceme in pooled over the environments, which suggested the existence of differences between parents and crosses for majority of characters in all

environments leading to evidence for manifestation of heterosis. The mean square due to check vs hybrids were significant for all the characters except, days to 50 % flowering in pooled over the environments.

Heterosis

An examination of performance of hybrids with respect to heterosis over better parent for seed yield/plant revealed that among 45 hybrids, 4 in E_1 , 8 in E_2 , 4 in E_3 and 7 in E_4 had significant heterobeltiosis which indicate the magnitude of heterobeltiosis was positive across the environments as few of the crosses exhibited positive heterotic effects. The heterobeltiosis for seed yield per plant range from -44.48 % (MCI 108 x SKI 337) to 30.61 % (SKI 341 x GC 3) in E_1 , -58.72 % (MCI 108 x SPS 35-50) to 39.98 % (SH 41 x SKI 337) in E_2 , -33.37 % (1379 x SKI 307) to 25.10 % (SKI 341 x GC 3) in E_3 and -48.64 % (MCI 108 x 1379) to 45.14 % (MCI 108 x GC 3) in E_4 respectively. The cross SKI 341 x GC 3 and SKI 341 x SPS 43-3 were gives consistence performance for seed yield per plant in all the environments. Over environments, heterobeltiosis for days to 50% flowering varied from -20.47 (GC-3 x 1379) to 32.86 % (MCI 108 x SKI-337) in E_1 , -13.1 (SKI-341 x 1379) to 36.23 (MCI 108 x SKI-337) per cent in E_2 , -14.62 (SKI-341 x 1379) to 36.57 (SKI-337 x SPS 43-3) in E_3 and -14.46 (GC-3 x 1379) to 34.85 (SKI-337 x SPS 43-3) per cent in E_4 respectively. The heterotic effects for days to maturity in negative direction are desirable for early harvest of the crosses. Out of 45 crosses, 8 in E_1 , 9 in E_2 , 9 in E_3 and 10 in E_4 exhibited significant negative heterobeltiosis for days to maturity. For this trait, the cross GC-3 x 1379 had possessed minimum significant heterobeltiosis in E_1 (-11.26 %), E_3 (-12.14) and E_4 (-12.26) whereas, in E_2 GC-3 x SKI-346 (-9.5 %) had the least estimates of heterobeltiosis. The less number of nodes up to primary raceme is desirable character to reduce the maturity. The crosses SKI-341 x SKI-346 depicted the least estimates of heterobeltiosis in E_1 (-14.04 %), SKI-341 x SH-41 has negative heterobeltiosis in E_2 (-6.69 %) and E_3 (-9.12 %) whereas GC-3 x 1379 had the minimum heterotic effect (-10.61 %) in E_4 for number of nodes up to primary raceme. For length of primary raceme crosses MCI-108 x SPS 43-3 (3.67 %), SKI 346 x SPS 35-50 (16.00 %), SH 41 x SPS 35-50 (3.54 %) and SPS 35-50 x SKI 307 (22.43 %) depicted the highest heterobeltiosis in E_1 , E_2 , E_3 and E_4 , respectively. For plant height up to primary raceme, the least estimates of heterobeltiosis were registered for cross MCI 108 x SKI-307 in E_1 (-28.65 %), whereas, in E_2 (-20.46 %) and E_4 (-18.63) cross GC 3 x SKI 307 and in E_3 SH 41 x SKI 337 (-12.15 %) had the lowest heterotic effect. For number of effective branches per plant, the cross SPS 35-50 x SPS 43-3 also depicted the maximum heterobeltiosis in E_1 (27.13 %); whereas, in E_2 (11.44 %), E_3 (12.35 %) and E_4 (7.63 %) cross SKI 341 x SH 41 had the highest heterotic effects, respectively. For number of capsules on primary raceme, the highest heterobeltiosis was depicted by crosses SH 41 x SKI 337 (17.02 %) in E_1 , 1379 x SKI 307 (67.53 %) in E_2 and (41.69 %) in E_4 , whereas, in E_3 , cross MCI 108 x SH 41 (40.68 %) exerted the highest heterobeltiosis. For test weight, among the 45 crosses evaluated, total 8 crosses in E_1 , 8 in E_2 , 13 in E_3 and 9 in E_4 depicted significant positive heterobeltiosis. The cross MCI 108 x SKI 307 exerted the maximum heterobeltiosis in E_1 (19.52 %), E_2 (18.43 %), E_3 (26.39 %) and E_4 (24.52 %) for test weight. For oil content, the cross SKI-346 x 1379 had the highest value of heterobeltiosis in E_1 (5.24 %); whereas, the cross GC 3 x SH 41 in E_1 (16.32 %) as well as E_2 (4.42 %) and GC 3 x SKI 307 in E_3 (9.91 %) had the maximum estimates of heterobeltiosis. For wilt incidence, the magnitude of heterobeltiosis were exerted by crosses SKI 341 x GC 3 in E_1 , SH 41 x SKI 337 in E_2 , SKI 341 x GC 3 in E_3 and cross MCI 108 x GC 3 in E_4 and that crosses which found for yield and its component viz., which also found superior to wilt resistance.

In the present study the magnitude of heterobeltiosis for seed yield/plant and no. of capsules on primary raceme was moderate to high. Similar finding was earlier reported by Ankineedu and Kulkarni, 1967; Varisaiet al., 1969; Patel,

1994a; Patel, 1998a and Joshi et al., 2001. Most of the yield components like days to 50% flowering, days to maturity, no. of total and effective branches/plant and length of primary raceme manifested moderate heterosis. Similar trends of heterobeltiosis were reported by Ankineedu and Kulkarni, 1967; Varisaiet al., 1969; Kaul and Prasad, 1983; Patel, 1989; Patel, 1994a; Tank et al., 2003 and Patel and Pathak, 2006. Present outcome of low heterobeltiosis for no. of nodes up to primary raceme and 100 seed weight was in agreement with earlier findings of Thakkar, 1987; Saiyed, 1993; Patel, 1994a, Tank et al., 2003 and Patel and Pathak, 2006.

Standard Heterosis

On commercial point of view, the superiority of newly developed hybrids should be judged by comparing their performance with the best cultivated hybrid/s or variety. Therefore the popular hybrids viz; GCH-7 of the region was used as standard check. For seed yield per plant hybrid MCI 108 x SKI 341 and SKI 341 x SPS 43-3 were exhibited significant positive standard heterosis in all the environments. Whereas, cross SKI 341 x GC 3 and MCI 108 x GC 3 were found significant positive heterotic effect at least in two environments. The crosses which found significant for seed yield per plant were also found resistance to wilt. The estimates of standard heterosis over GCH 7 (Check) ranged from -23.16 to 16.38, -14.62 to 18.13, -19.53 to 10.06 and -15.48 to 13.69 per cent in E_1 , E_2 , E_3 and E_4 , respectively for days to 50% flowering. For number of nodes upto primary raceme the cross SKI-341 x SKI-346 in E_1 (-16.95%), SKI-341 x SKI-337 in E_2 (-17.06 %) as well as E_3 (-25.16 %) and SKI-341 x SH-41 in E_4 (-10.77 %) had least estimate of standard heterosis. For length of primary raceme hybrid MCI 108 x SPS 43-3 had the highest estimate of standard heterosis in E_1 (15.31 %) and E_3 (15.88 %), respectively. For plant height hybrid SPS 43-3 x SKI 307 (18.08 %) expressed the maximum heterotic effect in E_1 and E_2 (24.11 %) and MCI 108 x SPS 43-3 in E_3 (25.29 %) and SPS 35-50 x SPS 43-3 in E_4 (30.42 %) exhibited the highest standard heterosis.

For number of capsules on primary raceme hybrid SKI 341 x SPS 43-3 (18.26 %) in E_1 , SKI 341 x GC 3 (22.55 %) in E_2 , MCI 108 x SPS 43-3 (21.68 %) in E_3 and SKI 341 x SH 41 (22.28 %) in E_4 . (Table 3)

The highest yielding hybrid MCI-108 x SKI-341 manifested significant and desirable heterosis over standard check GCH-7 for most of all the important yield contributing characters viz., length of primary raceme, number of internodes upto primary raceme, number of capsules on primary raceme and wilt incidence; This emphasized that high degree of standard heterosis for seed yield/plant might be due to the desirable heterosis observed for their important component traits. High association of heterosis between seed yield components and seed yield/plant in castor have also been earlier reported by Kaul and Prasad (1983), Patel et al; (1986), Thakkar (1987), Mehta et al. (1991), Patel (1991), Joshi (1993), Patel (1994b), Bhand (1996), Patel (1996), Patel (1998b), Manivelet al. (2000), Joshi et al. (2001) and Patel and Pathak (2006). Earlier Whitehouse et al; (1958) and Grafius (1959) suggested that there cannot be any gene system special for seed yield per se, as yield is an end product of multiplicative interaction of several yield components. Thus present investigation manifested that high magnitude of heterosis for seed yield/plant in castor is associated with high degree of heterosis in yield component traits. Number of capsules on primary raceme and 100 seed weight have significant influence on seed yield/plant followed by spike length and effective branches/plant.

Future Strategy

The hybrids viz; MCI-108 x SKI-341, SKI-341 x SPS 43-3, SKI-341 x GC-3, MCI-108 x GC-3 and SKI-341 x SH-41 registered significant and high magnitude of standard heterosis as well as wilt resistance over the environments thus

providing the best chances to isolate high yielding and earlier maturing genotype/s in later segregating generations. Bi-parental mating design between superior plants in segregating generations of these crosses will be fruitful to evolve high yielding genotypes having short stature, compact nodes, long spikes with more number of capsules and bolder seeds.

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APPENDICIES

Table 1: List of Parent Materials used for Present Study

Sr.No.	Name of Parental Lines	Pedigree/Parentage	Sources/Origin
1.	MCI 108	Mandore Selection	Mandore, Rajasthan
2.	SKI-341	Geeta x SKI 232	SDAU, Sardarkrushinagar
3.	GC-3	JP 65 x JI 88	JAU, Junagadh
4.	SH-41	VP 1 x SKI 7	SDAU, Sardarkrushinagar
5.	SKI-337	SKP 23 x SKI 215	SDAU, Sardarkrushinagar
6.	SKI-346	SKP 72 x SKI 232	SDAU, Sardarkrushinagar
7.	1379	Exotic culture (Israel)	DOR, Hyderabad
8.	SPS-35-50	Local collection	SDAU, Sardarkrushinagar
9.	SPS-43-3	Local collection	SDAU, Sardarkrushinagar
10.	SKI-307	SKI 112 x SKI 49	SDAU, Sardarkrushinagar

Table 2: Analysis of Variance for Different Traits under Pooled over the Environments in Castor

Source of Variation	Df	Days to 50 % Flowering	Days to Maturity	No. of Nodes upto Primary Raceme (cm)	Length of Primary Raceme (cm)	Plant Height upto Primary Raceme (cm)	No. of Effective Branches Per Plant	No. of Capsules On Primary Raceme	Seed Yield Per Plant (g)	100 Seed Weight (g)	Oil Content (%)	Wilt Incidence (%)
Environments	3	68.83**	178.47**	5.06**	102.02*	537.13**	28.18**	2281.98**	147389.07**	150.75**	8.40**	101498.77**
Replications	2	-	-	-	-	-	-	-	-	-	-	-
Genotypes	55	216.15**	837.24**	19.34**	1752.97**	1562.86**	21.20**	2146.85**	22954.71**	25.79**	81.56**	6139.49**
Parents (P)	9	305.13**	1310.45**	33.93**	1874.47**	2410.71**	24.14**	2682.90**	11539.79**	10.36**	78.52**	7057.27**
Hybrids (H)	44	202.79**	739.68**	16.80**	1756.84**	1424.16**	20.74**	2053.14**	25714.95**	27.84**	75.48**	5786.06**
P vs.H	1	2.91	871.14**	0.00	489.02**	35.35	14.86**	1445.24**	4238.10**	74.73**	376.56**	13430.73**
C vs. H	1	0.50	65.96**	7.33**	3471.93**	1232.54**	165.51**	3988.81**	39704.85**	117.76**	1.31	14819.10**
Error	110 (440)	3.58	8.06	0.67	32.72	28.77	1.17	38.48	344.64	0.77	0.83	7.33

Table 3: Manifestation of Heterobeltiosis (HB) and Standard Heterosis (SH) for Different Characters in Three Top Ranking Crosses for Seed Yield per Plant in E₁ Environment in Castor

Sr. No.	Name of Characters	E ₁					
		Heterobeltiosis (HB)			Standard Heterosis (SH)		
		SKI 341 x GC 3	SKI 341 x SPS 43-3	SKI 341 x SH 41	MCI 108 x SKI 341	SKI 341 x GC 3	SKI 341 x SPS 43-3
1	Days to 50% flowering	-5.36	18.45 **	-10.98 **	10.73 **	-10.17 **	12.43 **
2	Days to maturity	-8.19 **	8.81 **	-9.23 **	10.59 **	-7.65 **	12.65 **
3	No. of nodes upto primary raceme	-0.36	8.42 *	-5.63 *	4.75 **	-6.78 **	4.75 **
4	Length of primary raceme (cm)	-13.72	3.41	-19.02 *	0.00	-16.73 *	15.01 *
5	Plant height upto primary raceme (cm)	-5.94	7.92	12.63	-4.61 **	-23.37 **	-0.08 **
6	No. of effective branches per plants	12.92	-3.65	-2.53	-7.08	-18.14 **	-24.12
7	No. of capsules on primary raceme	-10.37	-2.19	12.91	-0.44	8.39	18.26 **
8	Seed yield per plant (g)	30.61 **	25.83 **	22.33 **	24.82 **	24.09**	20.69 **
9	100 seed weight (g)	-7.13 *	-5.31	-4.06	-12.07 **	-10.88 **	-10.21 **
10	Oil content (%)	-4.93 *	-8.30 **	1.65	6.24 **	-8.20 **	2.70
11	Wilt incidence (%)	0.00	0.00	0.00	0.00	0.00	0.00

* Significance at 0.05 per cent probability level, ** Significance at 0.01 per cent probability level

Table 4: Manifestation of Heterobeltiosis (HB) and Standard Heterosis (SH) for Different Characters in Three Top Ranking Crosses for Seed Yield per Plant in E₂ Environment in Castor

Sr. No	Name of Characters	E ₂					
		Heterobeltiosis (HB)			Standard Heterosis (SH)		
		SH 41 x SKI 337	SKI 341 x SPS 43-3	SKI 341 x GC 3	SKI 341 x SPS 43-3	MCI 108 x SKI 341	MCI 108 x GC 3
1	Days to 50% flowering	8.7 **	12.94 **	-2.94	12.28 **	16.37 **	0.58 **
2	Days to maturity	12.68 **	9.35 **	-6.82 **	13.86 **	8.85 **	-9.73 **
3	No. of nodes upto primary raceme	13.08 **	3.08	-1.06	0.67 **	2.34 **	-5.69 **
4	Length of primary raceme (cm)	2.45	-4.64	-2.13	3.44	-0.05	-7.58
5	Plant height upto primary raceme	0.00	14.65 *	4.09	7.08 **	0.08 **	7.78 **
6	No. of effective branches per plants	5.45	3.81	9.30	-10.83	-3.53	-9.82
7	No. of capsules on primary raceme	-9.00	19.08 **	33.03 **	9.70	10.36	15.42 *
8	Seed yield per plant (g)	39.98 **	33.43 **	29.22 **	29.52 **	28.30 **	24.37 **
9	100 seed weight (g)	9.81 **	-4.07	-6.03	-5.79	0.41	-1.10
10	Oil content (%)	-5.20 *	-11.26 **	-7.12 **	-1.70	0.55	-3.86
11	Wilt incidence (%)	0.00	0.00	0.00	0.00	0.00	0.00

Table 5: Manifestation of Heterobeltiosis (HB) and Standard Heterosis (SH) for Different Characters in Three Top Ranking Crosses for Seed Yield per Plant in E₃ Environment in Castor

Sr. No	Name of Characters	E ₃					
		Heterobeltiosis (HB)			Standard Heterosis (SH)		
		SKI 341 x GC 3	SKI 341 x SPS 43-3	SH 41 x SKI 337	MCI 108 x SKI 341	SKI 341 x SPS 43-3	SKI 341 x GC 3
1	Days to 50% flowering	4.14	4.60	10.45 **	6.51 **	7.69 **	4.14 **
2	Days to maturity	-5.07 *	9.54 **	12.72 **	6.91 **	13.81 **	-4.50 **
3	No. of nodes upto primary raceme	7.12 *	1.66	12.50 **	-0.65 **	-1.29 **	-2.90 **
4	Length of primary raceme (cm)	-15.43 *	1.76	-2.52	1.55	14.89 *	-16.53 *
5	Plant height upto primary raceme	-2.44	2.14	-12.15	15.82 **	2.31 **	-11.04 **
6	No. of effective branches per plants	6.96	6.07	8.33	1.01	-16.37 **	-7.05
7	No. of capsules on primary raceme	9.45	-2.24	-1.30	3.02	-12.20 *	-15.22 **
8	Seed yield per plant (g)	25.10 **	24.61 **	20.79 **	10.18	8.13	5.94
9	100 seed weight (g)	-4.37	-2.42	17.13 **	-0.40	-2.68	-6.04 *
10	Oil content (%)	-14.38 **	-10.61 **	4.76	-3.06	2.34	-1.97
11	Wilt incidence (%)	0.00	0.00	0.00	0.00	0.00	0.00

* Significance at 0.05 per cent probability level, ** Significance at 0.01 per cent probability level

Table 6: Manifestation of Heterobeltiosis (HB) and Standard Heterosis (SH) for Different Characters in Three Top Ranking Crosses for Seed Yield per Plant in E₄ Environments in Castor

Sr. No.	Name of Characters	E ₄					
		Heterobeltiosis (HB)			Standard Heterosis (SH)		
		MCI 108 x GC 3	MCI 108 x SKI 341	SKI 341 x GC 3	SKI-341 x SPS 43-3	MCI 108 x GC 3	MCI 108 x SKI 341
1	Days to 50% flowering	4.09	9.14 **	0.00	9.52 **	5.95 **	13.69 **
2	Days to maturity	-3.35	0.00	-5.49 **	15.73 **	-5.93 **	3.86 **
3	No. of nodes upto primary raceme	3.96	8.01 *	5.40	5.05 **	-2.69 **	4.38 **
4	Length of primary raceme (cm)	-0.69	7.68	-2.89	-8.89	-9.81	-1.06
5	Plant height upto primary raceme (cm)	19.54 **	7.41	-16.67 **	20.63 **	24.60 **	21.43 **
6	No. of effective branches per plants	-5.38	-4.36	5.31	-11.85 *	-8.89	-7.90
7	No. of capsules on primary raceme	10.38 *	5.89	-7.71	16.85 **	14.91 **	10.23
8	Seed yield per plant (g)	45.14 **	43.19 **	40.06 **	37.29 **	34.46 **	32.65 **
9	100 seed weight (g)	1.03	-6.87	-9.36 **	-8.58 *	-3.80	-10.41
10	Oil content (%)	-1.86	-7.26 **	-9.57 **	-0.63	-6.62 **	3.73
11	Wilt incidence (%)	0.00	0.00	0.00	0.00	0.00	0.00

*Significance at 0.05 percent probability level, **Significance at 0.01 percent probability level